

this invention. As shown in FIG. 34, the distribution of beads is a strong function of the amount of RNA bound to them, and association of increasing amounts of RNA with the beads produces progressively more diffuse (less concentrated) bead collection patterns. FIG. 34 shows RNA Effect On Bead Collection and Purification Efficiency. The indicated quantities of Rat Liver Total RNA were captured on 0.125  $\mu$ l of SpeedBeads and RNA was purified for quantitation. Diffuse bead collection patterns are associated with increased bead losses due to hydrodynamic drag. As expected, RNA purification yield drops from nearly 90% at 2  $\mu$ g to about 70% at 40  $\mu$ g. This phenomenon is not evident in bench control experiments (FIG. 28, FIG. 29, and FIG. 30). This phenomenon may be due to electrostatic repulsion of RNA. However the high salt concentration of 1 $\times$  Bead Binding Buffer (1.25M NaCl) may significantly shield such ionic effects. Another possibility is that RNA association renders beads "sticky", causing them to adhere to (for example) the PDMS membrane as they encounter it. This might then prevent beads from concentrating by "falling down" into the deeper parts of the membrane. As shown in FIG. 35 (left), bead distribution does not appear to be strongly dependent on bead quantity, as 0.5 $\times$  and 2 $\times$  beads also failed to concentrate. Interestingly however, as shown in FIG. 35 (right), RNA purification efficiency does appear to be a strong function of bead quantity, as 0.5 $\times$  and 2 $\times$  beads yielded less purified RNA. It is perhaps surprising that 1 $\times$  beads turned out to be optimal. FIG. 35 shows RNA Effect as a Function of Bead Quantity. Forty  $\mu$ g of Rat Liver Total RNA was captured on the indicated quantities of beads. 1 $\times$  beads is 0.125  $\mu$ l SpeedBeads. This quantity of beads was chosen early in the project based on observations suggesting that this is the maximum amount that can be efficiently captured in the BPump. These observations suggest, therefore, that decreased at 2 $\times$  beads may be due to RNA purification efficiency BPump overload. Decreased RNA purification efficiency at 0.5 $\times$  beads may be due to increased non-specific bead losses in the chip and/or increased bead dispersion due to either increased electrostatic repulsion or stickiness.

#### E. Enzyme Reaction

**[0161]** Ambion Message Amp III reactions were sequentially and progressively checked after each reaction step on-chip, as indicated in

**[0162]** FIG. 36.

**[0163]** Exp 1 (+K, all off-chip) served as a positive control for the standard Message Amp III kit. The products of Exps 2-5, in which increasing numbers of steps are carried out on-chip, are then be compared to Exp 1. aRNA quantity and quality was monitored by absorbance, gel electrophoresis, and capillary electrophoresis (Agilent BioAnalyzer), which were also used to characterize aRNA size distributions. Stratagene Universal Human Reference (UHR) RNA was used as starting material.

**[0164]** Exp 2: Reverse Transcription (RT) Reaction. The results of on-chip RT reactions are shown in FIG. 37. Chip and bench Bioanalyzer size distributions appear similar, and surprisingly, the yield from chip-based RT is higher than the bench control. This may be attributable to inadvertently extended RT incubation times for the chip-based reactions.

**[0165]** FIG. 37 shows Exp 1 (Bench Positive Control, K+) and Exp 2 (Chip, RT). BioAnalyzer and UV absorbance characterization. Approximately 415 ng of UHRR was used for bench positive control and chip-based RT reactions. Incubations were as follows: 42C/30 m (RT), 16C/60 m (SS), 65C/

10 m (Kill), and 40C/120 m (IVT). Note that reaction times are shorter than Message Amp III. Each sample was run twice on the BioAnalyzer.

**[0166]** Exp 3: Second-Strand (SS) Reaction. The results of on-chip RT and SS reactions are shown in FIG. 38. Chip and bench size distributions and yields appear similar.

**[0167]** FIG. 38 shows Exp 1 (Bench Positive Control, K+) and Exp 3 (Chip SS). BioAnalyzer, UV absorbance, and agarose gel characterization. Approximately 415 ng of UHRR was used for bench positive control and chip-based RT reactions. Incubations were as follows: 42C/30 m (RT), 16C/60 m (SS), 65C/10 m (Kill), and 40C/120 m (IVT). Note that reaction times are shorter than Message Amp III. Each sample was run twice on the BioAnalyzer. Lane A3 on the gel is a —RNA bench negative control, lane RNA is UHRR starting material.

**[0168]** Exp 4: In-Vitro Transcription (IVT) Reaction. The results of on-chip RT, SS, and IVT reactions are shown in FIG. 40. Chip and bench size distributions and yields appear similar. FIG. 40 shows Exp 1 (Bench Positive Control, K+) and Exp 4 (IVT). BioAnalyzer, UV absorbance, and agarose gel characterization. Approximately 230 ng of UHRR was used for bench positive control and chip-based RT reactions. Incubations were as follows: 42C/30 m (RT), 16C/60 m (SS), 65C/10 m (Kill), and 40C/120 m (IVT). Note that reaction times are shorter than Message Amp III. Each sample was run twice on the BioAnalyzer. Lane A3 on the gel is a —RNA bench negative control, lane RNA is UHRR starting material.

**[0169]** Exp 5: Purification. The results of on-chip RT, SS, IVT reactions and purification are shown in FIG. 41. Chip and bench size distributions appear similar, however chip yields were only about 50% of bench. This is likely attributable to inefficient chip-based purification due to bead loss.

**[0170]** FIG. 41 shows Exp 1 (Bench Positive Control, K+) and Exp 5 (RNA Purification). BioAnalyzer, UV absorbance, and agarose gel characterization. Approximately 310 ng of UHRR was used for bench positive control and chip-based RT reactions. Incubations were as follows: 42C/30 m (RT), 16C/60 m (SS), 65C/10 m (Kill), and 40C/120 m (IVT). Note that reaction times are shorter than Message Amp III.

**[0171]** Yields and amplification factors are summarized in the tables shown in

**[0172]** FIG. 39. In general, amplification factors and input amount are inversely related, as expected. Overall, the data show that enzyme reactions are efficiently carried out in the breadboard system.

#### F. Microarray Analysis

**[0173]** Bench- and chip-generated aRNAs were compared on Affymetrix U133 Plus 2.0 whole genome microarrays. The experiment was designed along the lines of the Microarray Quality Control (MAQC) study so that results could be compared to industry standards. Consistent with MAQC, amplified RNAs were generated from two different RNA inputs: Stratagene UHRR and Ambion Human Brain Reference RNA (HBRR). The design of the experiment is outlined in FIG. 42. After bench- or chip-synthesis, all aRNAs were fragmented with Ambion Message Amp III reagents for 30 minutes at 94C, and shipped to Expression Analysis on dry ice.

**[0174]** FIG. 42. Microarray Experimental Design. Four sets of three samples were generated: Bench (B) UHRR and HBRR, and Chip (C) UHRR and HBRR. Affy and TaqMan